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(71) Applicant *(for all designated States except US)*: **PHARMACIA & UPJOHN S.P.A.** [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BISSOLINO, Pierluigi** [IT/IT]; Via Roma, 36/2, I-27020 San Giorgio Lomellina (IT). **MANTEGANI, Sergio** [IT/IT]; Via Carlo Pisacane, 57, I-20129 Milan (IT). **ORZI, Fabrizio** [IT/IT]; Via Angera, 10, I-20125 Milan (IT). **JABES, Daniela** [IT/IT]; Via Costanza, 3, I-20146 Milan (IT). **ALZANI, Rachele** [IT/IT]; Via Garibaldi, 58, I-20025 Legnano (IT). **D'ANELLO, Matteo** [IT/IT]; Via Matteotti, 18, I-20026 Novate Milanese (IT). **PERRONE, Ettore** [IT/IT]; Via Aldo Moro, 44, I-20010 Boffalora Ticino (IT).

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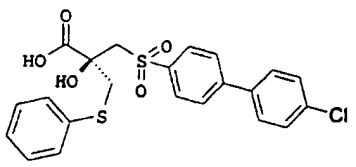
(54) Title: (R)-3-(4-CHLOROBIPHENYL-SULFONYL)-2-HYDROXY-2-(PHENYLTHIO)METHYLPROPIONIC ACID AND ITS USE MATRIX METALLOPROTEINASE

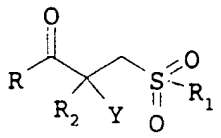


(Ia)

(57) Abstract: The present invention provides a compound which is (*R*)-3-(4-chlorobiphenyl-sulfonyl)2-hydroxy-2-(phenylthio)methylpropionic acid of formula (Ia) or a pharmaceutical acceptable salt thereof in enantiomerically pure form. The compound is an inhibitor of matrix metalloproteinases.

(R)-3-4(4-CHLOROBIPHENYL-SULFONYL)-2-HYDROXY-2-(PHENYLTHIO)METHYLPROPIONIC ACID AND ITS USE MATRIX METALLOPROTEINASE

This invention relates to an enantiomerically pure form of a α-hydroxy derivative of β-sulfonyl acid and its crystalline forms, to the pharmaceutically acceptable salts thereof, to a method of their preparation from the racemate, to the pharmaceutical compositions containing them, and to the method of using them. The compound of the invention acts as inhibitor of matrix metalloproteinases. The racemate compound was described in the PCT patent Application WO99/26909, that claimed compounds of the formula I:



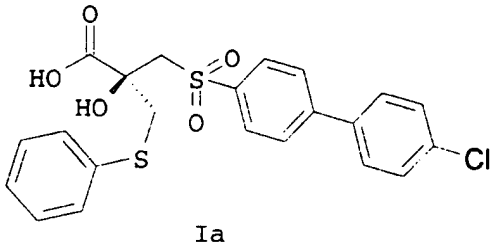
I

or pharmaceutical acceptable salts thereof wherein R is NHOH, R_1 is C_{4-12} alkyl, C_{4-12} alkenyl, C_{4-12} alkynyl, $-(CH_2)_h-C_{3-8}$ cycloalkyl, substituted and unsubstituted $-(CH_2)_h$-aryl, substituted and unsubstituted $-(CH_2)_h$-het, R_2 is substituted and unsubstituted C_{1-12} alkyl, substituted and unsubstituted C_{2-12} alkenyl, substituted and unsubstituted C_{2-12} alkynyl, substituted and unsubstituted $-(CH_2)_h-C_{3-8}$ cycloalkyl, substituted and unsubstituted $-(CH_2)_h-C_{3-8}$ cycloalkenyl, substituted and unsubstituted $-(CH_2)_h$-aryl, substituted and unsubstituted $-(CH_2)_h$-heterocyclic ring, substituted and unsubstituted $-(CH_2)_i-X-R_4$ (X=-O-, $-S(=O)_j-$, $-NR_7-$, $-S(=O)_2NR_8-$ or $-C(=O)-$), and $-(CH_2)_iCHR_5R_6$; Y is OH, NR_9R_{10}, or F. The corresponding intermediates having R=OH are also therein described. Even if in that application it was stated that the compounds of formula I contain a chiral center at the α-position of the hydroxamic or

carboxylic group, the only enantiomers specifically described are enantiomer A and B of N-hydroxy-2-hydroxy-2-[(4-methoxybenzenesulfonyl) methyl]-3-(4-phenylbenzenesulfonyl)-propionamide, prepared in Example 1 by separation with chiral chromatograhy.

We have now found that an enantiomer of a specific compound of formula I wherein R is OH has an interesting biopharmaceutical profile which surprisingly differs from that of the corresponding racemate, and a new method for its preparation starting from the corresponding racemate. Moreover, another object of this invention are the crystalline forms of said acid, that are particularly stable and suitable for pharmaceutical preparations.

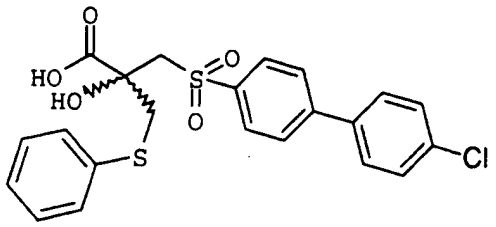
In a first aspect, the present invention relates to a compound which is (*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl]-propanoic acid of the formula Ia



Ia

or a pharmaceutically acceptable salt thereof, in enantiomerically pure form. The enantiomer has the (*R*) absolute configuration.

A further object of the present invention is the compound as defined above in crystalline form. Another object of the present invention is a process for preparing a compound of formula Ia by separation of the desired enantiomer from the corresponding racemate of the formula II



II

The desired enantiomer can thereby be obtained in constantly high yields and with high enantiomeric purity.

Description of figures

Figure 1. shows the powder X-ray diffraction spectrum of the acid compound prepared in example 3, with peak intensity (counts) as the vertical axis and angle (°2theta)as the horizontal axis.

X-ray diffractography (XRD)

The analyses were performed by irradiating powder samples with a Cukalfa graphite-monochromatic source by means of a computer controlled Siemens D-500 apparatus, performing a scan in the range between 5° and 35° (2theta) at room. The x-ray powder diffraction pattern of Figure 1 shows the crystalline structure of the compound prepared in example 3.

It has surprisingly been found that the severe demands imposed on the method to be developed are fulfilled with the use of a suitable optically active base such as (+)-ephedrine. The process of the present invention comprises treating a racemic compound of the formula II as defined above with (+)-ephedrine, (b) separating the resulting salt of the (*R*)-enantiomer of formula Ia by precipitation and (c) converting the separated salt into the free acid and, (d) if desired,converting the resulting free acid into a salt other than the salt with (+)-ephedrine.

The racemic acid compound of the formula II is particularly suitable as a starting material for the method according to the invention. The acid obtained according to well known methods (see SCHEME I) is treated with (+)-ephedrine, preferably in the presence of an

organic solvent, most preferably in the presence of an aliphatic C_1-C_5 alcohol. The salt of the (*R*)-enantiomer of the formula Ia which forms is separated, particularly by crystallisation, from the salt of the other enantiomer formed, and is subsequently isolated if desired by releasing the acid under acidic conditions and converted into a salt of a compound of the formula Ia which is different from that with ephedrine. The release of the acid of the compound of the formula Ia from the (+)-ephedrine salt can be effected with hydrochloric acid in a mixture of water and an organic solvent, for example ethyl acetate, methylene chloride, chlorofom, diethyl ether, diisopropyl ether, or 2-butanone. The compounds of the present invention can be converted to their salts, where appropriate, according to conventional methods.
For example, conversion of the compound of the formula Ia acid into the sodium salt can be effected with aqueous hydroxide. Analogously, other salts may be obtained by treatment with a suitable base in an appropriate solvent. The method according to the invention can be carried out economically and in an environmentally friendly manner.

The term "pharmaceutically acceptable salts" refers to acid addition salts useful for administering the compounds of this invention and include sodium, potassium, calcium and magnesium salts, and salts with pharmaceutically acceptable aminoacids such as L-arginine, L-lysine; and salts with other pharmaceutically acceptable bases, such as N-methylglucamine. These salts may be in hydrated form.

As stated before, the starting compounds for the method of preparation of the compound of the formula Ia of this invention can be prepared in accordance to the process depicted in Scheme I.

4'-Chloro[1,1'-biphenyl]-4-thiol, prepared according to GB1,121,722 or by methods well known to those skilled in the art, is reacted with 2-(bromomethyl)acrylic acid, commercially available. The resultant acrylic sulphanyl

derivative is then oxidized, converted to the corresponding methyl ester and then reacted with sodium hypochlorite. The resultant oxirane intermediate is readily condensed with thiophenol and hydrolyzed by procedures well known in the art such as saponification with aqueous alkali at 0° C to room temperature, to afford the desired racemic free acid of the formula II.

The pharmaceutical compositions of this invention may be prepared by combining the compound of formula Ia of this invention with a solid or liquid pharmaceutically acceptable carrier, and optionally, with pharmaceutically acceptable adjuvants and excipients employing standard and conventional techniques. Solid form compositions include powders, tablets, dispersible granules, capsules and suppositories. A solid carrier can be at least one substance which may also function as a diluent, flavoring agent, solubilizer, lubricant, suspending agent, binder, tablet disintegrating agent, and encapsulating agent. Inert solid carriers include magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, cellulosic materials, low melting wax, cocoa butter, and the like. Liquid form compositions include solutions, suspensions and emulsions. For example, there may be provided solutions of the compounds of this invention dissolved in water, water-propylene glycol, and water-polyethylene glycol systems, optionally containing conventional coloring agents, flavoring agents, stabilizers and thickening agents.

The pharmaceutical composition is provided by employing conventional techniques. Preferably the composition is in unit dosage form containing an effective amount of the active component, that is, the compounds of formula Ia according to this invention.

The quantity of active component, that is the compounds of formula Ia according to this invention, in the pharmaceutical composition and unit dosage form thereof may be varied or adjusted widely depending upon

the particular application method, the potency of the particular compound and the desired concentration. Generally, the quantity of active component will range between 0.5% to 90% by weight of the composition.

In therapeutic use for treating a patient, suffering from or susceptible to diseases involving matrix metalloproteinase activity, especially MMP-2 activity, the compounds or pharmaceutical compositions thereof will be administered orally, parenterally and/or topically at a dosage to obtain and maintain a concentration, that is, an amount, or blood-level of active component in the patient undergoing treatment which will be effective to inhibit such enzymes. In general, the active compound of formula (Ia) will be administered at dosages between about 0.1 and 30 mg/kg body weight of the subject to be treated per day, preferably from about 1 to 20 mg/kg given in a single dose or up to 3 divided doses. However, daily dosages can vary within wide limits and will be adjusted in each particular case according to the severity of the disease being treated, the requirement of the patient and the administration route.

Also, it is to be understood that the initial dosage administered may be increased beyond the above upper level in order to rapidly achieve the desired blood-level or the initial dosage may be smaller than the optimum and the daily dosage may be progressively increased during the course of treatment depending on the particular situation.

The present invention further provides the compound of formula (Ia) as defined above for use in a method of treatment or prophylaxis of diseases characterized by matrix metalloproteinase activity, especially when involving overexpression or activation of the gelatinase-A (MMP-2). Conditions particularly selected for treatment with the compound of formula (Ia) are cancer and metastatic diseases, optionally in combination with known cytotoxic agents. Other conditions where admnistration of the compound of formula (Ia) may prove advantageous

include restenosis, macular degeneration, and diseases characterized by formation of new blood vessels (neoangiogenesis).

Pharmaceutical compositions for parenteral administration will generally contain a pharmaceutically acceptable amount of the compounds according to formula Ia as a soluble acid addition salt dissolved in a pharmaceutically acceptable liquid carrier such as; for example, water-for-injection and a suitably buffered isotonic solution having a pH of about 3.5-6. Suitable buffering agents include; for example, trisodium orthophosphate, sodium bicarbonate, sodium citrate, N-methylglucamine, L(+)-lysine and L(+)-arginine, to name a few. The compounds according to formula Ia generally will be dissolved in the carrier in an amount sufficient to provide a pharmaceutically acceptable injectable concentration in the range of about 1 mg/ml to about 400 mg/ml. The resulting liquid pharmaceutical composition will be administered so as to obtain the above-mentioned inhibitory effective amount of dosage. The compound of formula Ia according to this invention is advantageously administered orally in solid and liquid dosage forms.

Biological Activity Test

The inhibitory activity of the compounds of the compound of formula (Ia) in comparison with reference MMP inhibitors was evaluated in vitro against human gelatinase-A (MMP-2) and human fibroblast collagenase (MMP-1) using the internal fluorescence quenching method. The assay method is based on the hydrolysis of the MMP substrate, MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 at 37 °C (see Knight, C.G. et al., FEBS Lett. 296:263-266, 1992). The enzymes cleave at the Gly-Leu bond removing the internally quenching DPA group. The release of the highly fluorescent peptide MCA-Pro-Leu was monitored with a LS50 Perkin Elmer fluorimeter equipped with a Peltier-thermostated cell holder using an excitation wavelength of 326 nm and an emission wavelenght of 398 nm. Proper

determination of steady-state rates of substrate cleavage required a preincubation at 37 °C of 15 minutes to allow for complete equilibration of the enzyme-inhibitor complex. For the determination of the apparent Ki ($Ki,_{app}$), the concentration of the inhibitor was varied at a constant and low concentration of the substrate (3 μM). Rates of substrate hydrolysis, expressed as fluorescence arbitrary units per minute, were calculated by least-square analysis of the steady-state linear progression curves. The steady-state rates were fitted to the Michaelis equation describing competitive inhibition; in particular, $Ki,_{app}$ were determined by fitting the data to the tight-binding equation of Morrison (Biochem. Biophys. Acta, 185:269-286, 1969) by non-linear methods. Since the Michaelis constants (K_m) for the MCA peptide substrate with the MMPs is quite high (70 μM or greater; see Knight, C.G. et al., reference above) and by far exceeds the substrate concentration, the factor ($1 + S/K_m$) can be approximated to unity without appreciable error, so that the determined $Ki,_{app}$ will be essentially equal to Ki.

Human gelatinase-A (MMP-2) was obtained as pro-enzyme (72 kDa) and was activated with 1 M 4-aminophenylmercuric acetate for 30 min at 37 °C immediately prior to use. The inhibitor was diluted into the assay from a stock solution in 100% DMSO, and controls substituted an equal volume of DMSO so that the final DMSO concentration from inhibitor and substrate dilution in all assays was 1%. The concentration of MMP-2 was 19.2 picoM. The assay buffer was 50 mM Tris/HCl pH 6.5 containing 150 mM NaCl, 10 mM $CaCl_2$, 0.01 mM $ZnCl_2$ and 0.05% Brij 35.

Human fibroblast collagenase (MMP-1) was obtained as truncated recombinant enzyme encompassing residues 101-269 and did not require activation. Assay conditions were the same as above, but enzyme concentration was 200 picoM. The Ki of the compound Ia on MMP-2 and MMP-1 were 9 and 7180 nM respectively. It is to be noted that the other enantiomer having configuration (S) showed a Ki >1000 nM

on MMP-2. The compound of formula Ia was found active by carrying out analogous biological test against other commercially availabe MMPs, in particular collagenase III (MMP-13), 40.5 nM and membrane type I (MMP-14), 192 nM.

Pharmacokinetic assay protocol

The pharmacokinetics and bioavailability of the compound of formula (Ia), sodium salt, were evaluated in male rats (Sprague Dawley CD) and in female Cynomolgus monkeys after administration of the drug at the single dose level of 15 mg/kg (corresponding to 14.32 mg/kg in free acid), PEG 400: 50 mM phosphate buffer pH 7.4 1:1 v/v as the vehicle. Three rats were dosed orally and other 3 intravenously. Three monkeys were treated orally and then intravenously after a washout period of 7 days. Blood samples were collected (from rats cannulated into the superior vena cava, in monkeys from the femoral vessels) into heparinized tubes at different times. The samples were immediately centrifuged (1200 g for 10 min at +4 °C) and stored at -80 °C pending analysis. The compound was extracted from plasma by protein precipitation by adding 200 µL of methanol to

25 µL of plasma. After mixing and centrifugation at 4000 rpm for 15 min, 10 µL of the supernatant was injected into the HPLC system. Mass spectrometric detection was by TurboIonspray using MRM in the negative ion mode. Pharmacokinetic calculations were performed using the non-compartmental approach (linear trapezoidal rule for AUC calculation) with the aid of WinNonLin (Scientific Consulting, Inc.).

The pharmacokinetic profile of compound Ia is summarized in the table below.

	Bioavailability	Plasma Clearance
Monkey	60%	0.02 ml/min/kg
Rat	27%	0.2 ml/min/kg

Safety.

The compound Ia showed no mortality, neither relevant clinical signs after a single oral dose of2000 mg/kg in rat and monkey; no treatment-related significant changes in hematology and clinical chemistry have been observed. In rats treated orally at doses up to 300 mg/kg/day for 15 days, no macroscopic clinical pathology or histology changes were observed in animals killed at the end of treatment. No clinical and histopathological signs of joint toxicity were observed at any tested dose.

Antitumor efficacy.

The antitumor efficacy of the compound Ia administered by oral route was determined in human tumor xenograft models in nude mice and in experimental models of metastasis. Results are summarized in the table below:

Experimental model	Dose/schedule	Efficacy
DU 145 human prostate ca.	100 - 200 mg/kg/day	44-56 % *
HCT 116 human colon ca	200 mg/kg/day	60 % *
A 375/M human melanoma metastasis	100 - 200 mg/kg/day	45-61 % **

* percent of tumor growth inhibition

** percent of reduction of number of metastasis

EXAMPLE 1

3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl]-propanoic acid (racemate)

Step 1. 2-{[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfanyl] methyl}acrylic acid

4'-Chloro[1,1'-biphenyl]-4-thiol (230 g; 1.04 mol) was added portionwise under stirring to a solution of α-bromomethylacrylic acid (190 g; 1.15 mol) in DMF (dimethylformamide; 1 L). The resulting solution was set aside for 20 min and then heated at 40 °C for 22 hours. The yellowish solution was poured into 7 L of deionized water and the resulting suspension was stirred for 1 h at rt. The precipitate was collected by filtration, washed

thoroughly with deionized water (1.5 L) and dried under vacuum at 50° C to a constant weight (about 327 g). This crude product was taken up in MBTE (methyl tert-butyl ether; 2 L) and the suspension was stirred for 1 h. The solid was collected by filtration and washed with MBTE (0.5 L) to afford 222.5 g (70 % yield) of the title compound as a white powder, mp 170-172 ° C.

^{1}H-NMR (400 MHz, DMSO-d_6); 3.85 (s, 2 H, CH_2S), 5.70 and 6.03 (two s, 2 H, $C=CH_2$), 7.41, 7.48, 7.61 and 7.67 (four d, J= 8.5 Hz, 8 H, aromatic protons), 12.80 (broad s, 1 H, COOH).

Step 2. 2-{[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl] methyl}acrylic acid

Oxone® (673 g) was added portionwise within 30 min to a stirred solution of 2-{[(4'-Chloro[1,1'-biphenyl]-4yl)sulfanyl]methyl}acrylic acid (222.5 g; 0.73 mol) in DMF (3.07 L) while maintaining the temperature in the range 30-35 °C by external cooling. After stirring for 2 h at 25-30° C, the solution was poured into 20 L of deionized water and the resulting suspension was stirred for 0.5 h. The precipitate was collected by filtration, washed with deionized water (12 L) and dried to afford 238 g (96.8 % yield) of the title compound as a white powder, mp 206-209 °C.

^{1}H-NMR (400 MHz, DMSO-d_6); 4.34 (s, 2 H, CH_2SO_2), 5.78 and 6.32 (two s, 2 H, $C=CH_2$), 7.57 (d, J= 8.5 Hz, 2 H, o-Cl aromatic protons), 7.79 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.86 (d, J= 8.5 Hz, 2 H, m-SO_2 aromatic protons), 7.94 (d, J=8.5 Hz, 2 H, o-SO_2 aromatic protons), 12.80 (broad s, 1 H, COOH).

Step 3. Methyl 2-{[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]methyl}acrylate

Cesium carbonate (214.6 g; 1.52 mol) was added portionwise within 20 min to a stirred solution of 2-{[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]methyl}acrylic acid (237 g) and methyl iodide (82.3 mL; 1.32 mol) in DMF (1.415 L). After stirring overnight, the cloudy solution

was poured into 10 L of deionized water and the resulting suspension was stirred for 1 h. The precipitate was collected by filtration, washed with deionized water (4 L) and dried to afford 246 g (99.6 % yield) of the title compound as a white powder, mp 139-140 °C.

^{1}H-NMR (400 MHz, DMSO-d_6); 3.58 (s, 3 H, CH_3O), 4.19 (s, 2 H, CH_2SO_2), 5.96 and 6.53 (two s, 2 H, $C=CH_2$), 7.46 (d, J= 8.5 Hz, 2 H, o-Cl aromatic protons), 7.54 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.71 (d, J= 8.5 Hz, 2 H, m-SO_2 aromatic protons), 7.92 (d, J= 8.5 Hz, 2 H, o-SO_2 aromatic protons).

Step 4. Methyl 2-{[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]methyl}-2-oxiranecarboxylate

Silica gel (171 g) was added to a stirred solution of methyl 2-{[(4'-chloro[1,1'-biphenyl]-4-yl)sulfonyl] methyl}acrylate (60 g; 0.171 mol) in 3 L of acetonitrile. To the resulting suspension, aqueous sodium hypochlorite (7% active chlorine titre; 311 mL) was added dropwise under vigorous stirring within 15 min. After stirring for 3.5-4.5 h, the silica was removed by filtration, and the filtrate was treated with 150 mL of 10 % sodium metabisulfite solution until disappearance of the oxidant. The solution was concentrated under reduced pressure at 30 °C, diluted with water (1 L), and extracted with ethyl acetate. The collected organic phase was washed with brine, dried and evaporated to leave a residue, which was taken up in MBTE (200 mL). The precipitate was collected by filtration and dried to afford 55 g (87 % yield) of the title compound as a white powder, mp 130-131°C.

^{1}H-NMR (400 MHz, DMSO-d_6); 3.07 and 3.22 (two d, J= 5.9 Hz, 2 H, oxirane CH_2), 3.72 (s, 3 H, CH_3O), 3.76 and 3.84 (two d, J= 4.6 Hz, 2 H, CH_2SO_2), 7.46 (d, J= 8.5 Hz, 2 H, o-Cl aromatic protons), 7.55 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.74 (d, J= 8.5 Hz, 2 H, m-SO_2 aromatic protons), 8.01 (d, J= 8.5 Hz, 2 H, o-SO_2 aromatic protons).

Step 5. Methyl 3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl] -2-hydroxy-2-[(phenylsulfanyl)-methyl] propanoate

Under a nitrogen blanket and stirring, finely ground potassium carbonate (3.1 g; 0.022 mol) was added to solution of methyl 2-{[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]methyl}-2-oxiranecarboxylate (57.2 g; 0.1559 mol) and thiophenol (17.5 mL; 0.17 mol) in DMF (400 mL). The reaction mixture was stirred for 30 min and than poured under stirring into 1400 mL of deionized water containing 100 mL of 1 N hydrochloric acid. The resulting mixture was extracted twice with ethyl acetate. The combined extracts were washed with brine, dried and evaporated to leave a residue, which was taken up in 200 ml of MTBE. The solid was collected by filtration, washed with MTBE (100 mL) and dried to afford 61.9 g (83.2 % yield) of the title compound as a white powder, mp 123-125 °C.

^{1}H-NMR (400 MHz, DMSO-d_6); 3.28 and 3.40 (two d, J= 13.6 Hz, 2 H, CH_2S), 3.44 (s, 3 H, CH_3O), 3.90 (s, 2 H, CH_2SO_2), 5.87 (s, 1 H, OH), 7.1-7.4 (m, 5 H, S-phenyl protons), 7.56 (d, J= 8.6 Hz, 2 H, o-Cl aromatic protons), 7.76 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.90 (s, 4 H, o+m-SO_2 aromatic protons).

Step 6

3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl] -propanoic acid

Aqueous 2 N sodium hydroxide (253 mL; 0.506 mol) was added to a solution of methyl 3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl) methyl]- propanoate (120 g; 0.251 mol) in DMF (2 L). After stirring for 1 h at 25-35 °C, the solution was acidified to pH 1-2 with 2 N HCl, then diluted with 6 L of deionized water and thoroughly extracted with ethyl acetate. The combined organic phase was washed with brine, dried and evaporated to leave a residue, which was taken up in 500 mL of MTBE. The suspension was stirred for 1 h, the solid

was collected by filtration, washed with MTBE and dried to afford 99.2 g (85 % yield) of the title compound as a white powder, mp 210-213°C.

^{1}H-NMR (400 MHz, DMSO-d_6); 3.33 (s, 2 H, CH_2S), 3.87 (m, 2 H, CH_2SO_2), 5.50 (broad s, 1 H, OH), 7.1-7.4 (m, 5 H, S-phenyl protons), 7.57 (d, J= 8.6 Hz, 2 H, o-Cl aromatic protons), 7.77 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.90 (s, 4 H, o+m-SO_2 aromatic protons), 13.40 (broad s, 1 H, COOH).

EXAMPLE 2

(+)-Ephedrine salt of (*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl]propanoic acid

(+)-Ephedrine (3.1 g; 0.0187 mol) and racemic 3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl]propanoic acid (8.7 g; 0.0187 mol), prepared as described in Example 1, were added to absolute ethanol (435 mL). The mixture was refluxed until complete solubilization, then left aside at room temperature for 48 h without stirring. After this time, the resulting suspension was stirred for 2 h. The precipitate was collected by filtration, washed with cold absolute ethanol (20 mL), and dried under vacuum at 45 °C until constant weight, thereby obtaining 6.1 g of the title product as a white powder, mp 178-180 °C.

Chiral HPLC Analysis (Column: Chiralpack AS, 0.46 x 25 cm; Mobile phase: ethanol with 0.1% TFA; Flow rate: 0.4 mL/min; Detection: U.V., 270 nm):

(*R*)-enantiomer (peak 1): 90.0 % (as area count)

(*S*)-enantiomer (peak 2): 9.9 % (as area count).

Optical rotation: $[\alpha]_D$ = -9.63° (c= 0.5 % in DMF)

The salt (6.1 g) obtained from the first crystallization was then dissolved in 600 mL of boiling absolute ethanol under stirring and the clear solution was allowed to cool spontaneously to room temperature for 48 h. The resulting suspension was cooled at 5-10 °C and

stirred for 1 h, then filtered. The solid was washed with 20 mL of cold absolute ethanol and dried to afford 5.4 g (45.76 % yield) of the title compound as a white powder, mp 185-186 °C.

Chiral HPLC Analysis (Column: Chiralpack AS, 0.46 x 25 cm; Mobile phase: ethanol with 0.1% TFA; Flow rate: 0.4 mL/min; Detection: U.V., 270 nm):

(*R*)-enantiomer (peak 1): 98.36 % (as area count)

(*S*)-enantiomer (peak 2): 1.53 % (as area count).

Optical rotation: $[\alpha]_D$ = -16.5° (c= 0.5 % in DMF)

EXAMPLE 3

(*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl) methyl]-propanoic acid

The pure (+)-ephedrine salt of (*R*)-3-[(4'-chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl]propanoic acid (5.4 g), prepared as described in Example 2, was suspended in a mixture of ethyl acetate and deionized water (60 mL each), cooled at 5° C, and treated dropwise with 2 N hydrochloric acid (7.8 mL). After stirring for 15 min at +5 °C, the organic layer was separated, washed with 60 mL of deionized water, dried and evaporated. The resulting residue was taken up in a mixture of MTBE/n-hexane (1:1; 30 mL), and the suspension was stirred at room temperature for 30 min. The precipitate was collected by filtration, washed with a fresh amount of the MTBE/n-hexane mixture (15 mL) and dried to afford 3.87 g of the title compound as a white solid (44.4 % overall yield from the racemate; 88.8 % of theory).

^{1}H-NMR (400 MHz, DMSO-d_6); 3.33 (s, 2 H, CH_2S), 3.87 (m, 2 H, CH_2SO_2), 5.50 (broad s, 1 H, OH), 7.1-7.4 (m, 5 H, S-phenyl protons), 7.57 (d, J= 8.6 Hz, 2 H, o-Cl aromatic protons), 7.77 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.90 (s, 4 H, o+m-SO_2 aromatic protons), 13.40 (broad s, 1 H, COOH).

Elemental analysis: Found, C 56.93 %, H 4.20 %, S 13.96 %.

Optical rotation: $[\alpha]_D$ = + 10° (c= 0.2 % in DMF).

DSC (Differential Scanning Calorimetry.): 164°C (form I);

167°C (form II).

EXAMPLE 4

Sodium salt of (*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenyl sulfanyl)-methyl]propanoic acid

Acqueous 1 N sodium hydroxide (155 mL; 0.155 mol) was added dropwise to a stirred solution of (*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenylsulfanyl)methyl]propanoic acid (67.5 g; 0.146 mol), obtained as described in Example 3, in 500 mL of THF and 2 L of absolute ethanol. After stirring for 30 min at 10 °C, the precipitate was collected by filtration, sequentially washed with 300 mL of absolute ethanol and 300 mL of diethyl ether, and dried to constant weight to provide 58.9 g (83.3 % yield) of the title compound as a white powder, DSC 186°C.

^{1}H-NMR (400 MHz, DMSO-d_6); 3.23 and 3.44 (two d, J= 12.5 Hz, 2 H, CH_2S), 3.63 and 3.70 (two d, J= 14.7 Hz, 2 H, CH_2SO_2), 5.23 (s, 1 H, OH), 7.0-7.3 (m, 5 H, S-phenyl protons), 7.55 (d, J= 8.6 Hz, 2 H, o-Cl aromatic protons), 7.77 (d, J= 8.5 Hz, 2 H, m-Cl aromatic protons), 7.86 (s, 2 H, m-SO_2 aromatic protons), 7.92 (d, J= 8.5 Hz, 2 H, o-SO_2 aromatic protons).

Elemental Analysis: Found, C 54.59 %, H 3.74 %, S 13.22 %, Na 4.74 %.

Optical rotation: $[\alpha]_D$ = - 89° (c= 0.2 % in DMF).

EXAMPLE 5

L-Arginine salt of (*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenyl sulfanyl)-methyl]propanoic acid

Operating as described in Example 4, but employing an aqueous solution of L-arginine instead of acqueous sodium

hydroxide, the title compound was obtained in 82% yield, m.p.100°-135°C.

^{1}H-NMR (400 MHz, DMSO-d_6): 1.5-1.7 (m, 4 H, arginine β- and γ-CH_2), 3.06 (m, 3 H, arginine α-CH and δ-CH_2), 3.22 and 3.41 (two d, J= 12.8 Hz, 2 H, CH_2S), 3.66 and 3.72 (two d, J= 14.9 Hz, 2 H, CH_2SO_2), 5.16 (broad s, 1 H, OH), 7.09 (m, 1 H, p-S aromatic proton), 7.23 (m, 2 H, m-S aromatic protons), 7.29 (m, 2 H, o-S aromatic protons), 7.3-8.6 (broad signals, 8 H, exchang. protons), 7.56 (d, J= 8.6 Hz, 2 H, o-Cl aromatic protons), 7.78 (d, J= 8.6 Hz, 2 H, m-Cl aromatic protons), 7.87 and 7.92 (two d, J= 8.5 Hz, o- and m-SO_2 aromatic protons).

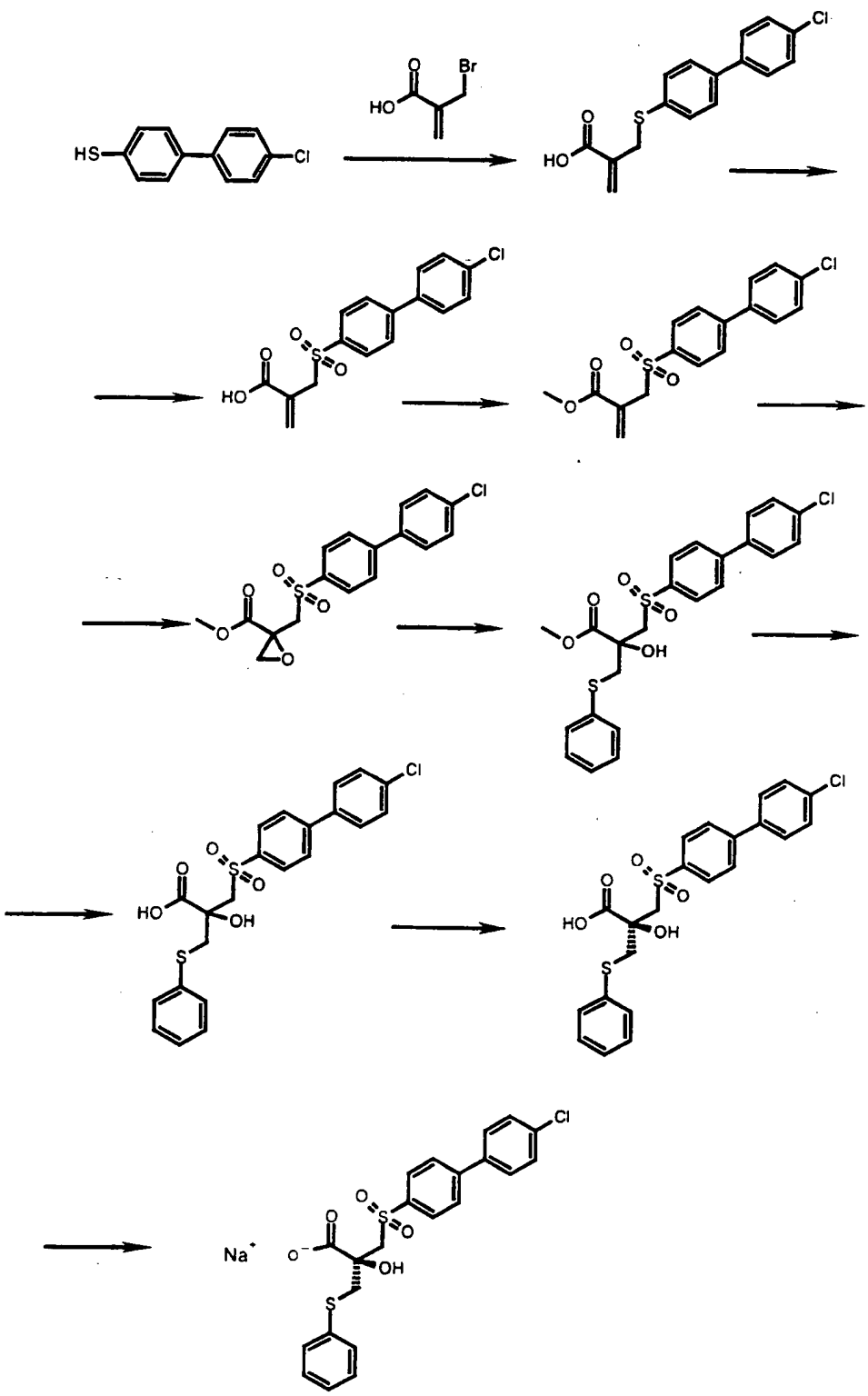
EXAMPLE 6

N-methylglucamine salt of (*R*)-3-[(4'-Chloro[1,1'-biphenyl]-4-yl)sulfonyl]-2-hydroxy-2-[(phenyl sulfanyl)-methyl]propanoic acid

Operating as described in Example 4, but employing an aqueous solution of N-methylglucamine instead of acqueous sodium hydroxide, the title compound was obtained in 80% yield, m.p. 50°-75°C.

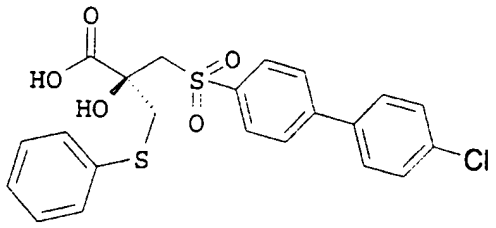
^{1}H-NMR (500 MHz, DMSO-d_6): 2.53 (s, 3 H, glucamine $NC\underline{H}_3$), 2.92 (dd, J= 9.6 and 12.7 Hz, 1 H, glucamine $C\underline{H}HNCH_3$), 3.02 (dd, J= 3.4 and 12.7 Hz, 1 H, glucamine $CH\underline{H}NCH_3$), 3.24 (d, J= 12.7 Hz, 1 H, $C\underline{H}HS$), 3.40 (m, 1 H, glucamine $HOC\underline{H}H$), 3.43 (d, J= 12.7 Hz, 1 H, $CH\underline{H}S$), 3.44 (d, J= 1.7 Hz, 1 H, glucamine $HOCH_2CH(OH)C\underline{H}OH$), 3.48 (m, 1 H, glucamine $HOCH_2C\underline{H}$), 3.59 (dd, J= 3.5 and 11.0 Hz, 1 H, glucamine $HOCH\underline{H}$), 3.65 (d, J= 14.8 Hz, 1 H, $C\underline{H}HSO_2$), 3.66 (dd, J= 1.7 and 5.1 Hz, 1 H, glucamine $HOCH_2CH(OH)CH(OH)C\underline{H}$), 3.73 (d, J= 14.8 Hz, 1 H, $CH\underline{H}SO_2$), 3.86 (m, 1 H, glucamine $HOCH_2CH(OH)CH(OH)CH(OH)C\underline{H}$), 4.4-5.4 (broad signals, 8 H, exchang. protons), 7.10 (m, 1 H, p-S aromatic proton), 7.23 (m, 2 H, m-S aromatic protons), 7.30 (m, 2 H, o-S aromatic protons), 7.57 (d, J= 8.7 Hz, 2 H, o-Cl aromatic protons), 7.79 (d, J= 8.7 Hz, 2 H, m-Cl aromatic protons), 7.88 and 7.94 (two d, J= 8.7 Hz, o- and m-SO_2)

SCHEME 1



CLAIMS

1. A compound which is (*R*)-3-(4-chlorobiphenyl-sulfonyl)-2-hydroxy-2-(phenylthio)methylpropionic acid of formula Ia



Ia

or a pharmaceutical acceptable salt thereof, in enantiomerically pure form.

2. A compound according to claim 1 which is crystalline.

3. A compound according to claim 2 which has a melting point of 164°C.

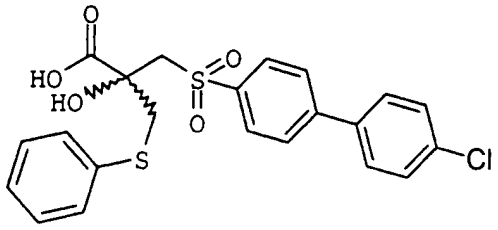
4. A compound according to claim 2 which has a melting point of 167°C.

5. A compound according to claim 1 which is the sodium salt of the acid of formula Ia.

6. A compound according to claim 1 which is the potassium, calcium, magnesium, N-methylglucamine, L-arginine or lysine salt of the acid of formula Ia.

7. A process for producing a compound as defined in claim 1, which process comprises

(a) treating a racemic compound of formula II:



with (+)-ephedrine,

(b) separating the resulting salt of the (*R*)-enantiomer of formula Ia as defined in claim 1 by precipitation,

(c) converting the separated salt into the free acid; and

(d) if desired, converting the resulting free acid into a salt thereof other than the salt with (+)-ephedrine.

8. A process according to claim 7, wherein the precipitation in step (b) is effected in the presence of an organic solvent.

9. A process according to claim 8 wherein the organic solvent is an aliphatic C_1-C_5 alcohol.

10. A method of treatment by therapy or prophylaxis of a disease characterized by matrix metalloproteinase activity, which method comprises administering to a patient in need thereof an effective amount of a compound as defined in claim 1.

11. A method according to claim 10 wherein the disease involves overexpression or activation of the gelatinase-A (MMP-2).

12. A method according to claim 11 wherein the disease is cancer or metastatic disease.

13. A method according to claim 11 wherein the disease is restenosis, macular degeneration or a disease characterized by the formation of new blood vessels (neoangiogenesis).

14. A method according to claim 10 wherein the said compound is administered alone or in combination with a known cytotoxic agent.

15. A method according to claim 10 wherein the said compound is administered orally, parenterally, or topically in an amount of from about 0.1 to about 30 mg/kg of body weight/day.

16. A pharmaceutical composition which comprises a pharmaceutically acceptable carrier or diluent and, as an active ingredient, a compound as defined in claim 1.

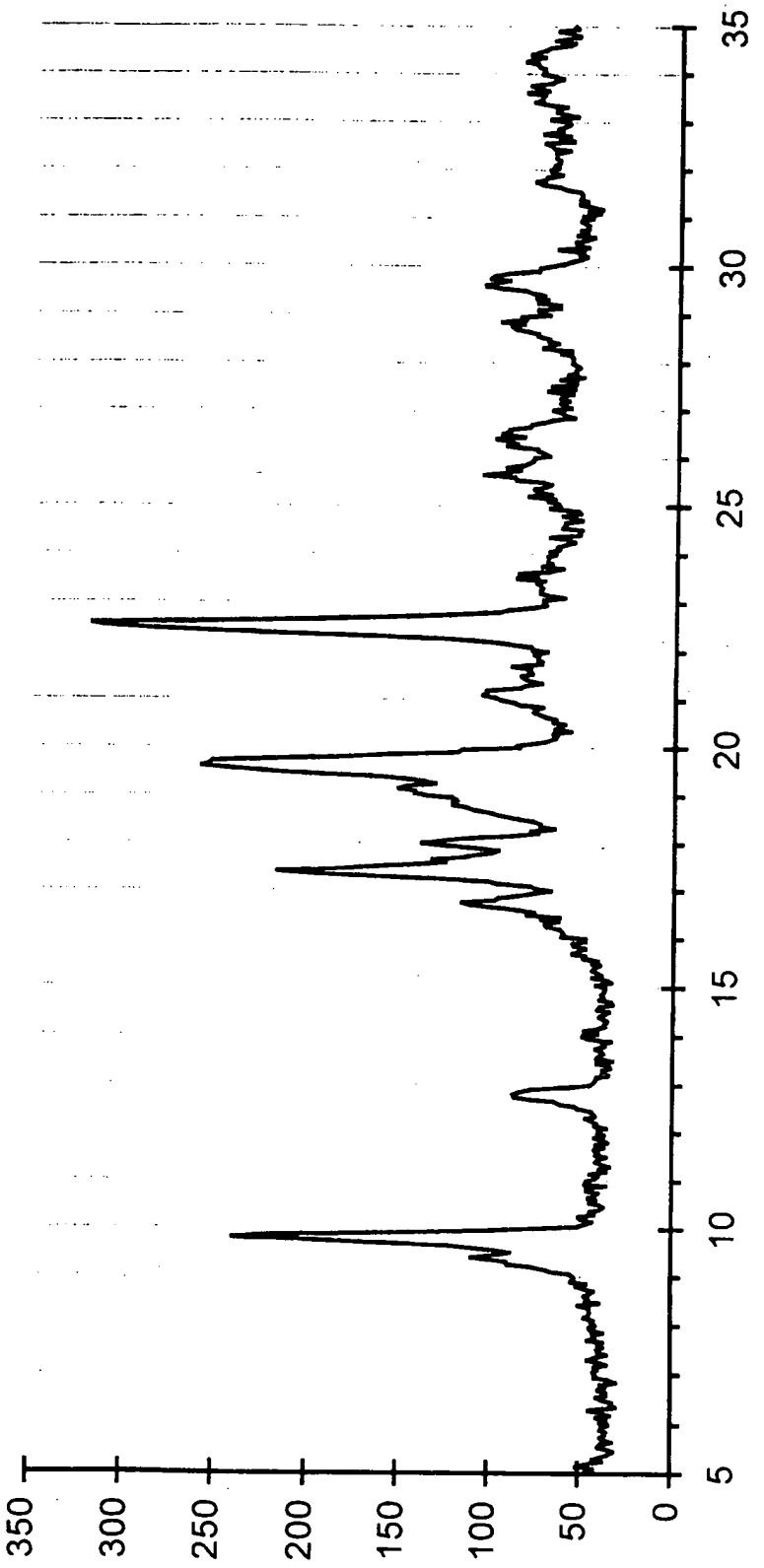
17. A compound as defined in claim 1 for for use as a medicament.

18. Use of a compound as defined in claim 1 in the manufacture of a medicament for the treatment by therapy or prophylaxis of a disease characterized by matrix metalloproteinase activity.

19. Use according to claim 18 wherein the disease involves overexpression or activation of the gelatinase-A(MMP-2).

20. Use according to claim 19 wherein the disease is cancer or a metastatic disease.

21. Use according to claim 19 wherein the disease is restenosis, macular degeneration or a disease characterized by the formation of new blood vessels (neoangiogenesis).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INTERNATIONAL SEARCH REPORT

Intern ...al Application No
PCT/EP 00/10837

A. CLASSIFICATION F SUBJECT MATTER
IPC 7 C07C323/65 A61K31/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 26909 A (PHARMACIA & UPJOHN CO.) 3 June 1999 (1999-06-03) cited in the application page 16, lines 14-23; page 17, line 7; page 42, line 16	1,7
A	WO 97 49679 A (ONO PHARMACEUTICAL CO LTD) 31 December 1997 (1997-12-31) abstract; page 228, example 23	10-21

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2001	29/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Amsterdam, L

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. ...al Application No
PCT/EP 00/10837

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		BR 9814699 A	03-10-2000
		EP 1037868 A	27-09-2000
		NO 20002505 A	30-06-2000
WO 9749679 A	31-12-1997	EP 0994104 A	19-04-2000